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Novel *CYP17A1* mutation in a Japanese patient with combined 17α-hydroxylase/17,20-lyase deficiency

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Abstract

Combined 17α-hydroxylase/17,20-lyase deficiency is caused by a defect of P450c17 that catalyzes both 17α-hydroxylase and 17,20-lyase reactions in adrenal glands and gonads. In the present study, we analyzed the *CYP17A1* gene in a Japanese girl with 17α-hydroxylase/17,20-lyase deficiency. The patient was referred to us for clitoromegaly at the age of 3 years. The karyotype was 46,XY. The patient was diagnosed as having 17α-hydroxylase/17,20-lyase deficiency based on the clinical and laboratory findings. Analysis of the *CYP17A1* gene revealed a compound heterozygous mutation. One mutation was a deletion of codon 53 or 54 encoding Phe (TTC) in exon 1 (ΔF54) on a maternal allele, which has been previously shown to partially abolish both 17α-hydroxylase and 17,20-lyase activities. The other was a novel missense mutation resulting in a substitution of Asn (AAC) for His (CAC) at codon 373 in exon 6 (H373N) on a paternal allele. Functional expression study demonstrated that the H373N mutation almost completely eliminates enzymatic activity. Previous studies have demonstrated that replacement of histidine by leucine at position 373 causes complete loss of both 17α-hydroxylase and 17,20-lyase activities with a defect in heme binding due to a global alteration of P450c17 structure, indicating the importance of H373 for P450c17 structure and function. Together, these results indicate that the patient is a compound heterozygote for the ΔF54 and H383N mutations and that these mutations inactivate both 17α-hydroxylase and 17,20-lyase activities and give rise to clinically manifest combined 17α-hydroxylase/17,20-lyase deficiency.

1. Introduction

Adrenal and gonadal 17α-hydroxylase/17,20-lyase (P450c17) catalyzes 2 sequential reactions: 17α-hydroxylation of pregnenolone or progesterone and 17,20-bond scission of the 17α-hydroxylated products [1]. The *CYP17A1* gene encoding this enzyme is located on chromosome 10q24.3 [2]; and more than 50 mutations in the *CYP17A1* gene have been reported to cause combined 17α-hydroxylase/17,20-lyase deficiency or isolated 17,20-lyase deficiency [3-6]. Adrenal 17α-hydroxylase deficiency is characterized by impaired production of cortisol and compensated hypersecretion of adrenocorticotropin, which stimulates the synthesis of large amount of deoxycorticosterone and corticosterone, leading to hypertension, hypokalemia, and a suppressed renin-angiotensin system. Gonadal 17,20-lyase deficiency prohibits the synthesis of dehydroepiandrosterone, testosterone, and estrogen, resulting in 46,XY disorders of sex development in genetic males and sexual infantilism in both sexes. Combined 17α-hydroxylase/17,20-lyase deficiency is a rare form of congenital adrenal hyperplasia, which accounts for about 1% of cases overall [7]; and isolated 17,20-lyase deficiency is extremely rare [8].

In the present study, we report a Japanese patient with combined 17α-hydroxylase/17,20-lyase deficiency and a novel *CYP17A1* mutation.

2. Materials and methods

2.1. Patient

The patient was a Japanese girl born to healthy and unrelated parents after an uneventful 40-week pregnancy.

This genetic study was approved by the Institutional Ethical Review Board at the National Center for Child Health and Development, and informed written consent for genetic analyses was obtained from each subject.

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The patient had a sibling, a healthy elder sister. The patient weighed 2980 g at birth. The patient was noticed to have clitoromegaly and was referred to our hospital at the age of 3 years and 6 months. The patient was 98.0 cm (+0.3 SD) in height and 15.5 kg (+0.5 SD) in weight. The patient had female external genitalia with a slightly enlarged clitoris. The blood pressure was 138-90/80-50 mm Hg. Serum Na and K were 144 and 5.5 mEq/L, respectively. The karyotype was 46,XY. Endocrinologic findings are summarized in Table 1. Plasma adrenocorticotropic hormone (ACTH) was marginally elevated. Plasma renin activity was suppressed, but occasionally rose to the reference range. Serum levels of 17-deoxysteroids such as progesterone and deoxycorticosterone were elevated and increased in response to ACTH stimulation, whereas serum levels of 17-hydroxysteroids such as 17-hydroxyprogesterone and cortisol increased only minimally. Serum testosterone increased after human chorionic gonadotropin (hCG) administration, but the response was blunted. At 5 years of age, laparoscopy revealed right inguinal testis, left intraabdominal testis, and blind-ending vagina (about 2 cm deep) with no uterus; and the patient underwent clitoroplasty and resection of the left intraabdominal testis. From 6 to 8 years of age, the patient was put on hydrocortisone therapy, but gave up taking medicine. At 13 years of age, the right testis was removed. At 15 years of age, the patient was commenced on estrogen therapy. The patient has been almost normotensive (120-86/51-89 mm Hg) and normokalemic (3.4-4.2 mEq/L) without glucocorticoid therapy.

These clinical and laboratory findings were compatible with a diagnosis of partial combined 17α-hydroxylase/17,20-lyase deficiency.

2.2. Analysis of the CYP17A1 gene

The genetic study was approved by the Institutional Ethical Review Board at the National Center for Child Health and Development. The genomic DNA of the patient and her parents was isolated from whole blood by proteinase K digestion and phenol/chloroform extraction after written informed consent for genetic analyses was obtained from each subject. All the 8 exons and exon-intron boundaries of the *CYP17A1* gene were amplified as described previously [9]. The amplified polymerase chain reaction products were fractionated and isolated on a 1% agarose gel (Bio-Rad Laboratories, Richmond, CA) and directly sequenced using a Thermo Sequenase kit (GE Healthcare Bio-Sciences, Piscataway, NJ).

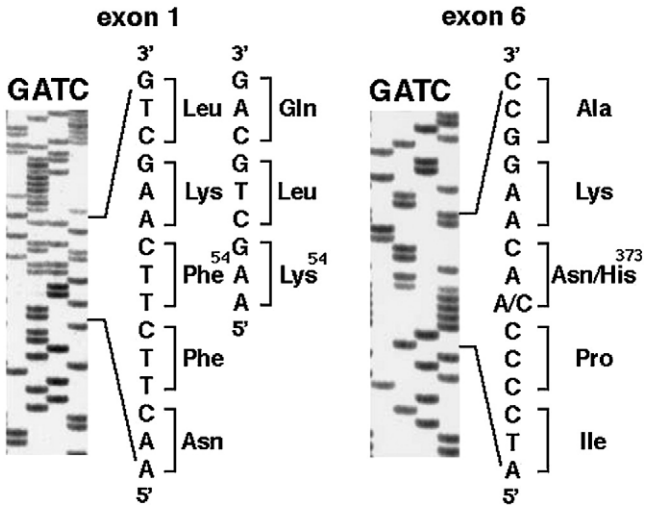

Fig. 1. Direct sequencing analysis of the *CYP17A1* gene from the patient. The patient has a heterozygous deletion of codon 53 or 54 (Δ54F) in exon 1 and a heterozygous missense mutation H373N in exon 6.

2.3. Functional expression study of the wild-type and mutant P450c17

The wild-type P450c17 complementary DNA (cDNA) was kindly provided by Dr Toshihiko Yanase (Fukuoka University, Fukuoka, Japan) [10]. The H373N substitution was introduced in the wild-type P450c17 cDNA by the recombinant polymerase chain reaction method; and the wild-type and H373N mutant P450c17 cDNAs were cloned into an expression plasmid pRK 5 as described

Table 1
Endocrinologic findings of the patient

	At 3 y old		At 11 y old	
	Basal	Post-hCG	Basal	Post-ACTH
ACTH (pg/mL)	17.3 (7.4-55.7)		121 (7.4-55.7)	
PRA (ng/[mL h])	1.64 (0.3-5.4)		0.1 (0.3-5.4)	
Aldosterone (pg/mL)	218 (28.8-371)		71 (79.3-209)	
Testosterone (ng/dL)	18.6 (2.0-25.9)	66.4 (182-298)		
Progesterone (ng/mL)			7.6 (0.13-0.93)	10.9 (0.84-2.04)
17-OHP (ng/mL)	0.2 (0.04-1.14)		0.3 (0.14-0.69)	0.4 (1.15-1.97)
DOC (ng/mL)			0.78 (0.09-0.34)	1.4 (0.33-1.39)
Corticosterone (ng/mL)			10.1 (2.34-13.7)	
Cortisol (μg/dL)			4.9 (5.7-15.0)	7.4 (22.0-27.0)

Post-hCG refers to the value after intramuscular injection of 4000 U/m^2 hCG for 3 days. Post-ACTH refers to values at 60 minutes after intravenous administration of 0.25 mg/m^2 ACTH. In the parentheses, reference values are shown. PRA indicates plasma renin activity; 17-OHP, 17α-hydroxyprogesterone; DOC, deoxycorticosterone.

previously [9], which were designated as pRK-17α and pRK-H373N, respectively.

COS-1 cells (RIKEN Cell Bank, Tsukuba, Japan) were transfected by electroporation (Gene Pulser II; Bio-Rad Laboratories, Hercules, CA) with 2 μg of pRK-17α, pRK-H373N, or pRK 5 only and 1 μg of pRK-GH1, a human growth hormone expression plasmid, which was used as an internal control of transfection efficiency. The cells were suspended in Dulbecco modified Eagle medium (Gibco BRL Life Technologies, Rockville, MD) containing 10% fetal calf serum and transferred to 35 × 10–mm FALCON tissue culture dishes (Nippon Becton Dickinson, Tokyo, Japan). Twenty-four hours after transfection, 5 μg/mL of progesterone (Sigma Chemical, St Louis, MO) was added to the media. After another 24 hours of incubation, the media were collected. The amount of 17α-hydroxyprogesterone and androstenedione in the media was determined simultaneously by high-performance liquid chromatography as previously described [9], and that of human growth hormone was determined by an immunoradiometric assay kit (Daiichi Radioisotope Laboratories, Tokyo, Japan). The experiments, each performed in triplicates, were carried out 4 times; and data are shown as mean ± SEM.

3. Results

The patient had 2 heterozygous mutations (Fig. 1). One was a deletion of codon 53 or 54 (TCC) encoding Phe in exon 1 (ΔF54). The other was a C to A transversion of the first nucleotide in codon 373 in exon 6, which resulted in a substitution of Asn (AAC) for His (CAC) at codon 373 (H373N). No other mutations were found in the entire exons and the exon-intron boundaries of the patient's *CYP17A1* gene. The mother was heterozygous for the ΔF54 mutation, and the father was heterozygous for the H373N mutation (data not shown). Therefore, it was concluded that the patient is a compound heterozygote bearing the ΔF54 mutation on a maternal allele and the H373N mutation on a paternal allele.

To determine the functional consequence of the H373N mutation, we transiently expressed the wild-type and H373N mutant P450c17. The COS-1 cells transfected with the wild-type P450c17 cDNA efficiently converted progesterone to 17α-hydroxyprogesterone and androstenedione, whereas those transfected with the empty pRK 5 plasmid failed to convert progesterone to 17α-hydroxyprogesterone and androstenedione (Table 2). The H373N substitution resulted in markedly reduced production of 17α-hydroxyprogesterone (0.2% of the wild-type P450c17) and no production of androstenedione (Table 2).

Table 2
17α-Hydroxylase/17,20-lyase activity of wild-type and mutant P450c17 expressed in COS-1 cells

Plasmid	17α-Hydroxyprogesterone (ng/dish)	Androstenedione (ng/dish)
pRK-17α	6100 ± 130	118.5 ± 21.6
pRK 5	<1	<1
pRK-H373N	15.0 ± 0.5	<1

Data are shown as mean ± SEM from 4 independent experiments.

4. Discussion

The ΔF54 mutation in exon 1 was first described in a Japanese female patient with partial combined 17α-hydroxylase/17,20-lyase deficiency who presented with hypertension, hypokalemia, and irregular menstruation [10]. Functional expression analyses revealed that the ΔF54 mutation partially reduces the activities of both 17α-hydroxylase and 17,20-lyase [10,11]. The ΔF54 mutation has been repeatedly identified in Japanese patients with combined 17α-hydroxylase/17,20-lyase deficiency [10-15], suggesting that this mutation has been derived from a single founder gene.

The H373N mutation in exon 6 has not previously been reported [3-6]; thus, this mutation appears to be a novel mutation. The functional expression study demonstrated that the H373N mutation results in almost complete loss of enzymatic activity. Thus, it is highly likely that the H373N mutation and the ΔF54 mutation are the causes of combined 17α-hydroxylase/17,20-lyase deficiency in our patient. Of note, another mutation involving codon 373, H373L, has been described in Japanese patients with combined 17α-hydroxylase/17,20-lyase deficiency by us and others [9,14-17] and is demonstrated to disturb heme binding and completely abolish both 17α-hydroxylase and 17,20-lyase activities by in vitro expression studies [16]. Computer modeling of human P450c17 suggests that H373, located in a β-sheet, forms a hydrogen bond with the carboxylate of E391 in the adjacent strand of the β-sheet; and thus, the H373L mutation creates a global alteration of P450c17 structure that secondarily prohibits heme binding [18]. Our novel H373N mutation, substituting the highly conserved basic histidine residue to a neutral asparagine residue, reconfirms the importance of the H373 residue for P450c17 structure and function.

In patients with combined 17α-hydroxylase/17,20-lyase deficiency, the elucidation of molecular basis generally explains the patient's clinical profiles [4,5]. The ΔF54 mutant P450c17 is shown to retain 10% to 23% 17α-hydroxylase activity and 5% to 12% 17,20-lyase activity [10,11]. Given that the H373N mutation almost completely abolishes both 17α-hydroxylase and 17,20-lyase activities, the residual activities of the ΔF54 mutant may, at least in part, account for the presence of clitoromegaly and the absence of hypertension and hypokalemia in our patient. To our knowledge, 6 patients with combined 17α-hydroxylase/17,20-lyase deficiency bearing the ΔF54 mutation at least in 1 allele have been reported in detail; 4 patients (1 genetic male and 3 genetic females) are homozygous for the ΔF54

mutation, and 2 patients (1 genetic male and 1 genetic female) are compound heterozygous for the ΔF54 mutation and the H373L mutation [10,12-15]. Blood pressure is described in 5 patients [10,12,13,15]; all of them are hypertensive in contrast with our patient. The external genitalia in the genetic male patient with the homozygous ΔF54 mutation are characterized by hypospadias and cryptorchidism [12]. The genetic male patient with the compound heterozygous ΔF54 and H373L mutation is reported to have female-type external genitalia with no ambiguity [14], whereas our patient has clitoromegaly. In a large-scale study from Brazil, substantial variations in blood pressure and potassium levels as well as in genital differentiation in genetic males can be observed among individuals with the same mutant *CYP17A1* [19]. Therefore, it is deduced that environmental and other genetic factors than *CYP17A1* genotype are also responsible for the clinical presentations of combined 17α-hydroxylase/17,20-lyase deficiency. Recently, it has been demonstrated that the *POR* gene encoding P450 oxidoreductase (POR), through which all microsomal P450 enzymes receive electrons from reduced nicotinamide adenine dinucleotide phosphate (NADPH), is very polymorphic and that an A503V variant POR affects the 17α-hydroxylase and 17,20-lyase activities of P450c17 [20]. Thus, it is possible that polymorphisms of the *POR* gene modify phenotype of combined 17α-hydroxylase/17,20-lyase deficiency; and this possibility can be tested in the large cohort of Brazilian patients with the same mutant *CYP17A1* [19].

In conclusion, we have demonstrated a novel genetic lesion in the *CYP17A1* gene that leads to combined 17α-hydroxylase/17,20-lyase deficiency.

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